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High-throughput epitope identification for snakebite antivenom

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Introduction
Insight into the epitopic recognition pattern for polyclonal antivenoms is a strong tool for accurate prediction of antivenom cross-reactivity and provides a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in 966 individual toxins from pit vipers (Crotalidae) using the ICP Crotalidae antivenom. Due to an abundance of snake venom metalloproteinases and phospholipase A₅ in the venoms used for production of the investigated antivenom, this study focuses on these toxin families.

Objectives
- Identify epitopes in toxins used in immunization
- Characterize tolerated amino acid substitutions in identified epitopes
- Predict cross-reactivity of antivenom

Epitopes locate to surface regions
To identify epitopes the observed peptide specific signal intensities were mapped back to the amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides with median signals above 20 AU, epitope core sequences were localized and subsequently mapped to crystal structures or homology models. As examples, P-I metalloproteinase and lyso49-phospholipase A₅ from Bothrops asper (venom used in antivenom production) are presented here.

Effect on cross-recognition
The α-helical right-handed secondary structure is conserved among pit viper toxins sharing at least seven of the eight epitope residues and mean signal intensities of the eight 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition. Binding is still observed in all of the corresponding eight 15-mer peptides, although the intensity of the eight 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition.

Conclusions
- Custom-designed high density peptide microarray technology enables parallel automated identification of epitopes in hundreds of toxins.
- Integrating multiple sequence alignment allows investigation of the effect of epitope variation on antivenom recognition.
- Cross-reactivity of antivenom is correlated to the degree of conservation in toxin epitopes and flanking residues.

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